

OCULAR DEVELOPMENT AND INVOLUTION IN THE EUROPEAN CAVE SALAMANDER, *PROTEUS ANGUINUS LAURENTI*

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Proteus anguinus Laurenti is a well-known urodele amphibian, and it is the only real cave-dwelling vertebrate in Europe. Its biology remains, however, a relatively unknown field of study. In spite of the interest it has aroused in naturalists, there remain many contradictions and inexactitudes in studies of which this urodele has been the object. These contradictions are found in many basic questions concerning the biology of the animal, its method of reproduction, its immature or larval state, and its adaptation to underground life.

On this last point, the genesis of the rudimentary state of the eyes (see Fig. 1) is of special interest. After two centuries of research, only a very general idea has been formed of the eye of *Proteus*. It is known that it is sensitive to light. The fact remains, however, that the precise forms of ocular development are unknown, and the problem of its involution remains unsolved. Two working hypotheses emerge from the contradictory accounts of previous research: the first is that the degeneration of the eye is a secondary consequence of the adaptation to an underground environment, with a link between the disappearance of the eye and the darkness in the caves; and the second is that the ocular atrophy is a kind of arrested development, linked to a larval neotenic state.

Scarcity of material explains this situation. It is not yet known where *Proteus* reproduces naturally. Ecologically, it is known that *Proteus* comes to feed in the underground rivers of the Dinaric Alps Karst. The water-bearing Karst, where its development takes place, remains inaccessible. This present work was only possible after cultures of *Proteus* had been established in the laboratory.

MATERIALS AND METHODS

A breeding culture of *Proteus* was begun at the underground laboratory of the C.N.R.S. in 1957 by Professor Vandel and his assistants. From observations on this breeding culture, it is known that oviparity is regular and that the development and growth of each animal continues until it reproduces in its turn around the fourteenth year (Vandel and Durand, 1970). There is no true metamorphosis, and each specimen of *Proteus* can be considered to be a mature animal (Fig. 2a). It has also been possible with the breeding cultures to study and experiment upon hundreds of specimens of embryos, larva, and young.

There is no need to set out the now classic histological or electron microscope methods, such as the silver impregnation of Bodian, nor to describe in detail methods of biometry, preparation of graphic reconstruction, or of the translucencies to examine skeletal parts, all of which can be found in many works.

The experiments consisting of exposure to daylight and to artificial light were carried out in aquariums where the water was kept under the same conditions

as the water where the control animals were found. The lighting was at levels of intensities between 100 and 6000 lux. The range of artificial lighting was between 800 and 1200 nm.

In all that is relative to the cultures, transplants, regeneration baths and hormone injections, the classic methods of experimental embryology were employed for the organotypic cultures and the xenoplastic transplants; the embryos used were at the tail-bud stage. From many species, two (*Pleurodeles waltlii* Michaelles and *Euproctus asper* Dugès) gave particularly satisfactory results and were retained as hosts or as donors. The animals were sacrificed from a period of a few days to a year and a half after each experiment.

RESULTS

DEVELOPMENT OF SENSE ORGANS

Organogenesis of the eye

As is the case with all vertebrates, one can distinguish the appearance of the presumptive eye field of *Proteus* at the time when the ectoblast is differentiated into epiblast and neuroblast. At the neurula stage (stage: 30–45 days, 6–7 mm at 11.6° C; Durand, 1971) the presumptive visual field comprises a part of the neural crests and a portion of the floor of the medullary groove. At the end of neurulation (45–50 days, 7–8 mm), the head is formed and the optic vesicles evaginate from the proencephalic region of the neural tube.

At the tail bud stage (50–55 days, 8–9 mm; Vandel, Durand and Bouillon, 1964), the optic vesicle is greatly enlarged and is joined to the ectoderm.

Between 60–70 days of development (10–11 mm), the lenticular placode is differentiated from the basilar epidermis, and the primary optic vesicle is transformed into a secondary optic cup. One notices the incipient dissociation between the single layer of cells of the future pigmented layer and the thickening of the retinal layers. At the anterior limb bud stage (70–80 days, 11–13 mm) where the lens is still attached to the basal epidermis, the retinal layer is deeply refolded (Fig. 2b). At the following stage (80–90 days, 13–15 mm) the lens which has separated from the ectoderm appears in the form of a closed epithelial vesicle in which the cellular nuclei are situated at the periphery.

At the stage of the cylindrical limb bud (90–100 days, 15–17 mm), the organization of the eye and its attendant envelopes is developed. One can see the organization of the retina and the optic nerve is differentiated. The corneal ectoderm is reduced to two layers of cells. At the time of the formation of the fingers of the anterior limb (stage 100–110 days, 17–18 mm), the inner plexiform layer of the retina appears. The lens differentiates at a slower rate than the retina when compared to other salamanders (*Pleurodeles*, *Euproctus*, *Ambystoma*). This is somewhat anomalous morphogenetically.

Between 110 and 120 days (19–24 mm), slightly before hatching, the eye pigment appears as a black semi-circle. The cerebral plexiform layer of the neural retina is spread out and divided into multipolar and bipolar cells. The visual cells appear and the lumen of the lens is rejected towards the surface, while the cells of the posterior pole begin to form a nucleus. At this stage, the embryo

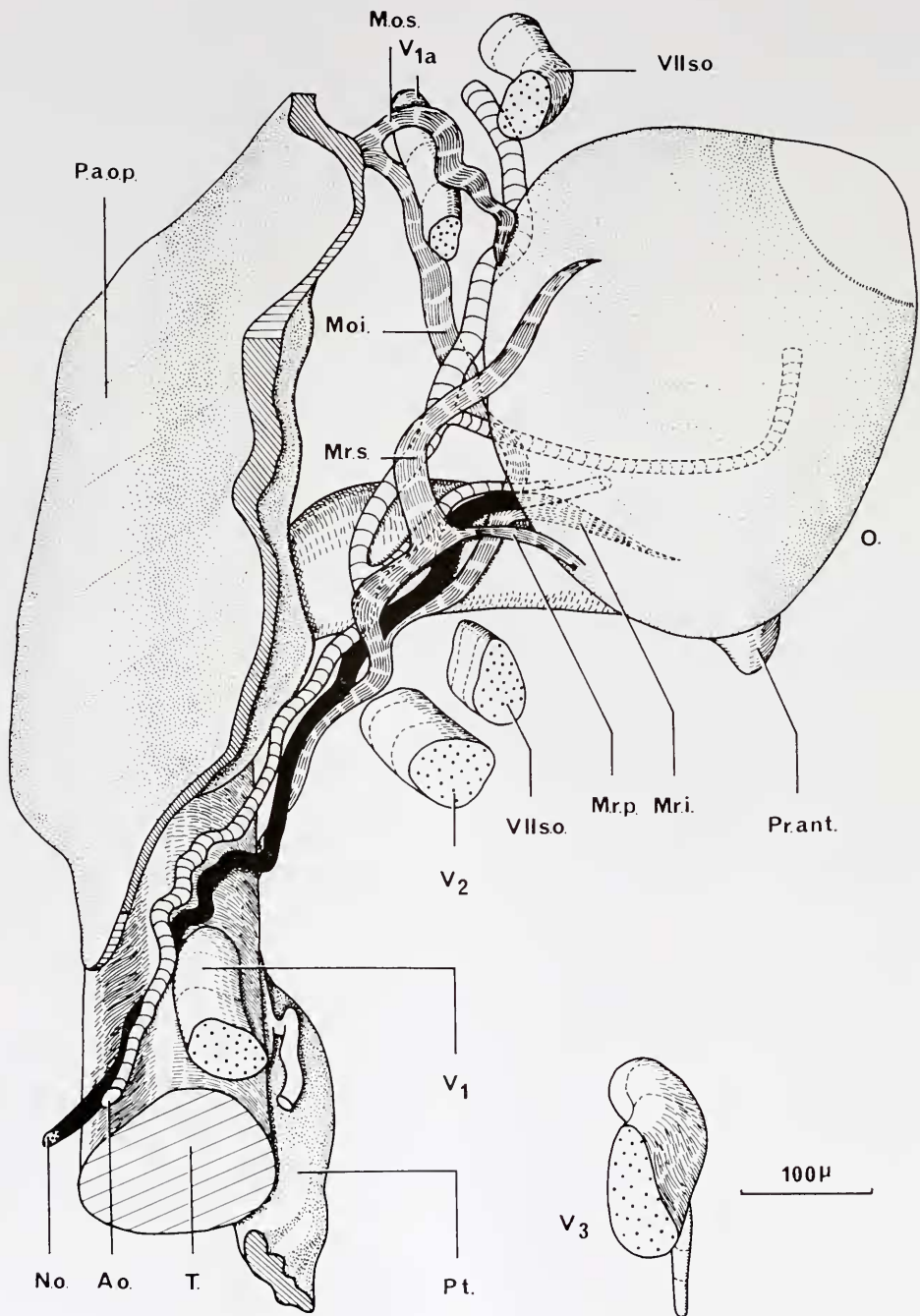


FIGURE 1. Larval orbit in *Proteus anguinus* prepared from sections by graphic reconstruction. P.a.o.p. represents the *processus orbito-parietale*; M.o.s., the *musculus obliquus superior*;

is transformed into a larva (hatching). From the exterior the gross appearance of the eye is circular and black. At its center, the clear spot of the lens is transparent. The eye is near to the state of its maximum differentiation (Fig. 2c). The external plexiform layer separates and the cells of the conjunctiva extend from the sclera in a primitive sclero-corneal limbus. The cornea remains essentially in a "dermoid cornea" state.

On the course of larval life (24-40 mm, 16-120 days after hatching), the eye grows but does not undergo further differentiation. A characteristic regressive development is the thickening of the supraoptic ectoderm (Fig. 2d) and the appearance of lytic vacuoles in the lens tissue (Fig. 3b and 3c).

At four months post-emergence, the larval stage ends. The individual enters the juvenile phase of development, but the eye will always retain its generally embryonic appearance which continues in the adult animal (Fig. 2e).

Analysis of ocular organogenesis

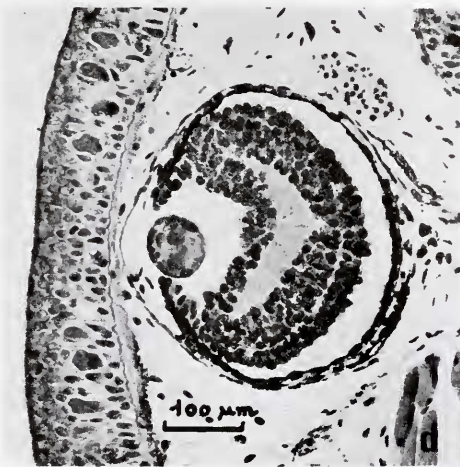
The modes of ocular organogenesis have proved to follow the normal sequence of embryogenesis which runs: archencephalic induction; evagination of the optic vesicle from the primitive diencephalon; transformation into the secondary optic cup; formation of a lens from the superficial ectoderm; and finally, differentiation of a thin and transparent dermal cornea. Such development would appear commonplace, if it were not for certain specific modifications, including the slowing up of some growth and anatomical migration and involution.

Slackening of growth. From the stage when the external plexiform layer and the expansion of the visual cells appear, the retina retains its single embryonic appearance (Fig. 2c): its elements are fewer in number and larger in size, the lens remains enclosed within the retina, the vitreous body hardly develops and the continuous fold between the retina and pigmented epithelium persists without giving rise to an iris.

Contrary to certain hypotheses, development does not stop. The retina continues to grow: the number of cells increase from 1500-2000 to more than 10,000 over a ten-year period; the pigmented epithelium retains its ability to regenerate a lens throughout the life of the animal; the cartilaginous plates of the sclera appear late (at about 3 or 4 years); and finally comparison of the first stage of organogenesis between the eye of *Proteus* and the eye of *Necturus*, another Proteidae, show that the development of the optic field of *Proteus* is very slow.

Anatomical migration. The eye progressively sinks into the cephalic tissues, and then connective tissue becomes interposed between the eye and the hypodermis. The eye is subject also to a retrocaudal displacement (Fig. 1). Starting anterior to the anteorbital process of the trabecula, it later comes to occupy a posterior position in the adult.

V_{1a}, the *ramus ophthalmicus profundus* of the Vth cranial nerve; VII_{s.o.}, the *ramus ophthalmicus superficiale* of the VIIth cranial nerve; M.o.i., the *musculus obliquus inferior*; M.r.s., the *musculus rectus superior*; O., the eye; Pr.ant., the *processus antorbitalis*; M.r.i., the *musculus rectus inferior*; M.r.p., the *musculus rectus posterior*; V₂, the *ramus maxillaris* of the Vth cranial nerve; V₁, the *ramus ophthalmicus* of the Vth cranial nerve; Pt., the *ossa pterygoidea*; V₃, the *ramus mandibularis* of the Vth cranial nerve; T., the *trabecula cranii*; A.o., the *arteria ophthalmica*; and N.o., the *nervus opticus*.



Involution. Principally, the involution of the dermal cornea is expressed by the supraocular ectoderm becoming thick and opaque (Fig. 2d and 2e); the involution of the lens is by enantiometric allometric growth which can lead to its disappearance (Fig. 4). Thus, the study of ocular development shows the growing dissociation which exists between the fate of the derivatives of the neuroblast and those derived from the epiblast of the eye.

The initial development would appear morphogenetically normal, if the slowing of growth was not manifested (starting from the closure of the neural ridges), and if some involutive phenomena did not appear at the time of the formation of the lens. These processes are reinforced during larval development, directing the eye towards its specific modifications which vary from the classic development of the vertebrate eye.

Correlation with other sense organs

After having described the ocular development of *Proteus*, it is useful to compare its characteristics with those of the other sense organs. The organization of the olfactory apparatus is simple, and it is well developed in the adult. Only the chondrification of the olfactory capsule shows a delay comparable to that for the chondrification of the cartilaginous plates of the scleral skeleton. In contrast to the development of the eye, the development of the olfactory sac itself is normal.

The comparison between the development of the acoustic apparatus of *Proteus*, and the development of the ear of the other amphibians shows that the organogenesis of the ear is somewhat delayed (for example, it requires 1800 hours at 12° C, against 275 hours at 10° C for *Rana sylvatica*). The primary developmental stages of the eye follows the sequence of ocular development, but the later development of the ear is clearly more rapid because the internal ear is well differentiated and the otic capsule chondrified at the end of the larval stage.

It is known that the lateral line organs register vibrations of the liquid environment in which the animal is immersed. The neuromasts of *Proteus* are of a superficial type and the lateral neurosensory system corresponds to the basic type for aquatic vertebrates.

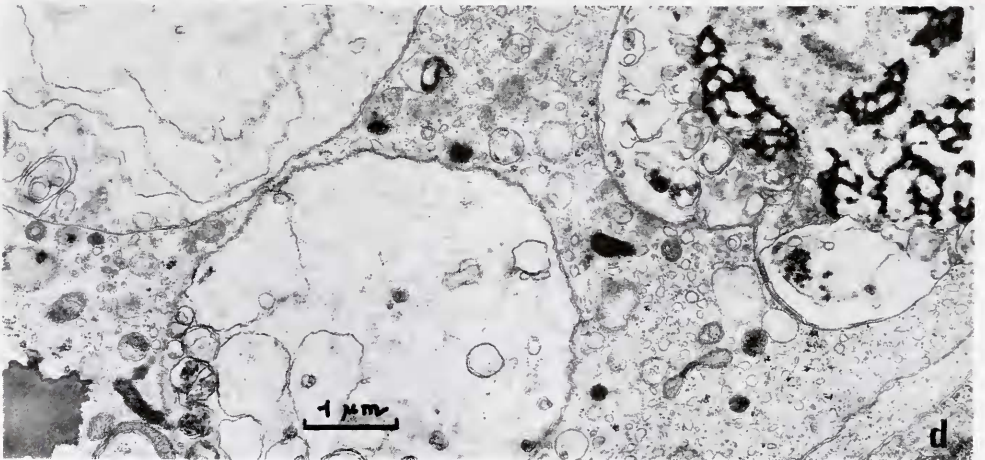
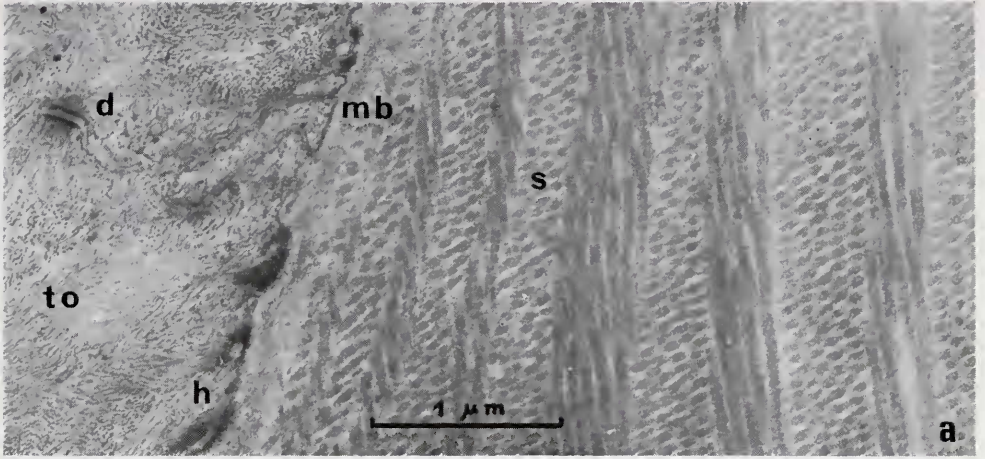
In summary, the development of sense organs of *Proteus* is slow and simple, but remains perfectly normal. One can say that the initial development of the eye follows a normal course, but that its development is extremely slow and the organ cannot attain the normal structure of the vertebrate eye. Development begins with a marked ontogenetical slowing, and finally is clearly regressive.

DEVELOPMENT OF LARVAL, JUVENILE AND ADULT EYE

Cephalic organization

Skeleton. The cranium of *Proteus* is more elongated and less massive than those of the other urodeles. One can see the stability of the chondrocranium,

FIGURE 2. a: Adult *Proteus*; b, a microphotograph of the eye at the anterior limb bud stage; c, the eye at the hatching stage; d, the eye at the larval stage; and e, the eye at the adult stage.



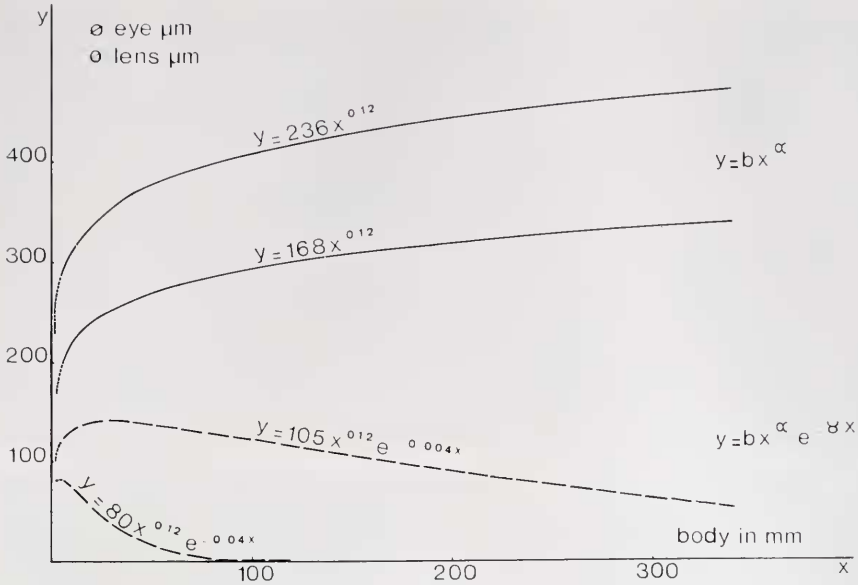


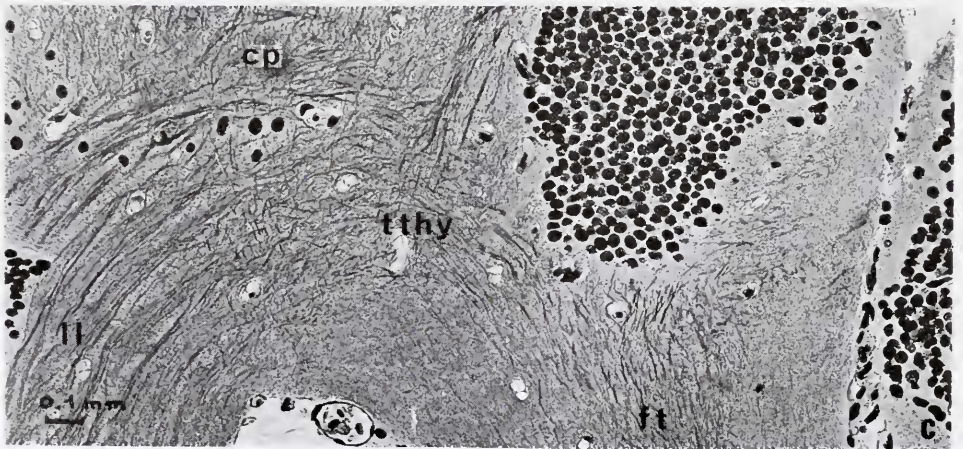
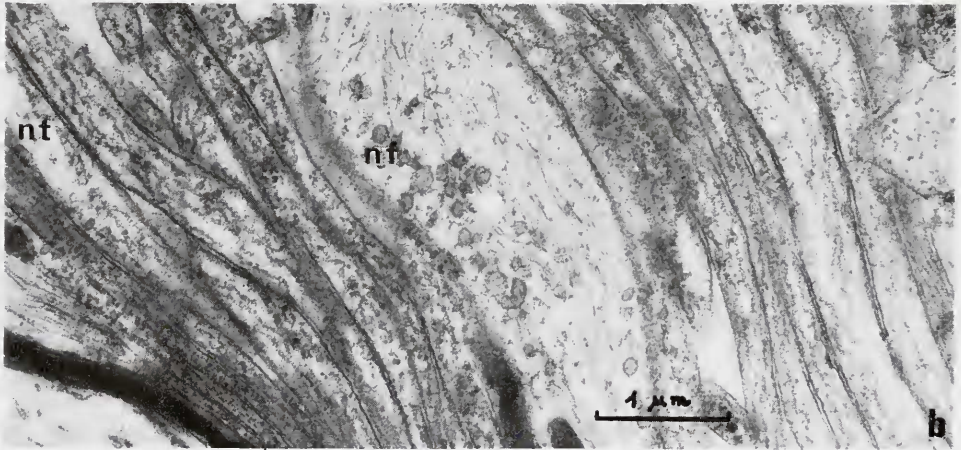
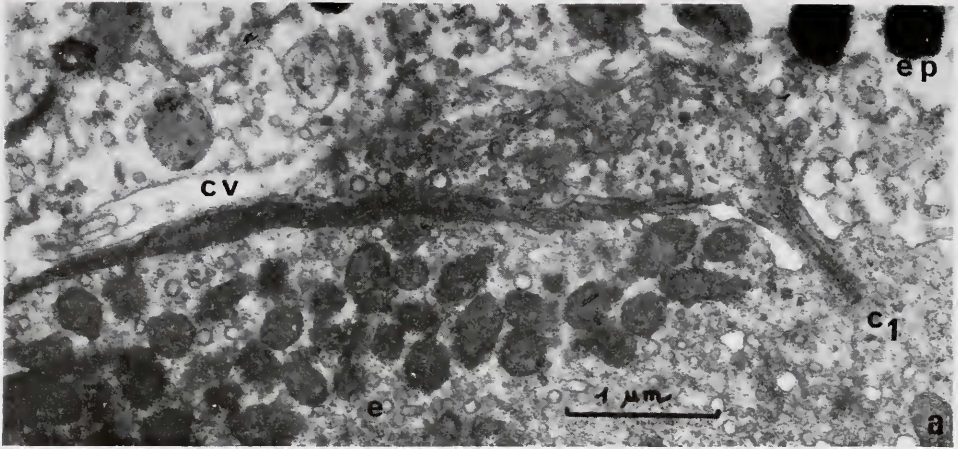
FIGURE 4. Maximal and minimal values for growth of the eye and of the lens in *Proteus* in relation to the body size. Units of the ordinate are microns, and of the abscissa body length in millimeters. Solid lines and the accompanying equations are those for the relative growth of the eye; broken lines, those for the lens.

except in the nasal and orbital region where the olfactory capsules show only slight development. The scleral cartilage is frequently absent, and the orbito-sphénoïd is usually absent. In general, the anterior part of the orbital cartilage is ossified and constitutes a large part of the walls of the cranium. Because of the missing skeletal pieces in *Proteus*, one observes an extension of the parietal bone to the trabecula. Such evidence from study of the skeleton, in particular the hypobranchial skull being only partially ossified, along with the low sensitivity of tissues to thyroidal hormones, does not support the view of other authors (Hawes, 1946) in hypothesizing a neotenic state, but indicates that the skeletal organization of *Proteus* is fundamentally simple.

Musculature. The muscles of the orbit are numerous, and can be grouped: the cephalic muscles into elevators and depressors of the jaw, and the muscles of the buccal floor, deep muscles and dorso-longitudinal muscles (Durand, 1971).

Nervous system. The brain of *Proteus* corresponds to the classic brain of amphibians such as *Necturus*. It presents, however, some differences: a greater elongation; its position in the posterior zones of the cranium, by the development of the olfacto-gustative regions; and the smaller development of diencephalic and mesencephalic regions. It is important to note that, contrary to certain opinions, the auricular cerebellum exists, and the acoustico-lateral area is predominant in

FIGURE 3a. Dermoid cornea by electron microscopy: d, dendrite; mb, basal membrane; s, stroma; h, hemidesmosome; to, tonofibril. 3b shows larval lens; c, electron micrograph of the degenerative lens; d, electron micrograph of the lens lytic vacuoles.



the medullary centers. Lastly, the branches of the cranial nerves, with the exception of those of the trochlear (IV) and abducens (VI) can be followed.

In general, the observed simplicity concerning the cephalic organization as well as the organization of the other apparatus inclines one to think that *Proteus* is not a neotenic form, as is often said, but rather a perennibranch. This can only confirm the interpretation of Vandel (1966) who considers this animal as a relict form surviving underground.

Orbital attachment

Variations of the extrinsic musculature of the eye lie within the corresponding limits of the embryonic stages which precede the complete differentiation of these muscles. One can show a clear parallel between the state of development of the ocular muscles (reduced or absent, Fig. 1), and the weak development or the disappearance of the ocular motor nerves, and the reduction of vascularization. The elongation of the ocular muscles, of the nerves of the orbit, of the optic nerves and of the ophthalmic vessels is tied to the great elongation of the cranial structures and the relatively minor development of the diencephalic nervous system. In this connection, they indicate that throughout all of the juvenile life of the animal, said to extend through a dozen years, marked disharmonies of growth can be observed between the diverse parts of the body of *Proteus*.

Ocular tissues

Dermoid cornea. The cornea of *Proteus* is made up of two elements: a part of the original epithelium and part of the original sclera. In the electron micrograph (Fig. 3a) of the cells of the corneal epithelium, one notices the hemidesmosomes of the dermo-epithelial type connecting Bowman's membrane and the stroma to regularly arranged collagenous fibers. The scleral part of the cornea is comprised principally of fibroblasts. They delimit an anterior chamber of the eye which encloses a very finely granular material, the vitreous humor. The supraocular tegument of the larva of *Proteus* is differentiated in a transparent dermoid-cornea, comparable to that of some cyclostomes, Dipnoi and *Ichthyophis*.

Lens. The differentiation proceeds as in the case of the other vertebrates, but does not pass the stage of the embryonic lens at the time when the fibers and the primitive nucleus are formed. For example, in a lens of 100 μm diameter (Fig. 3b), one notices that the anterior epithelium is composed of monostratified cuboidal cells, the primitive lens fibers, and the large nucleus.

The pycnotic figures should be noted along with the regions of degeneration which accompany cellular lysis (Fig. 3c), and intranuclear vacuoles. In fact, the rudiment of lens cells encloses a large number of lytic vacuoles (Fig. 3d) or autophagic vacuoles which correspond to the degeneration centers.

FIGURE 5. Electron micrographs of a: the visual cells of the adult (ep represents pigmented epithelium; cv, visual cell; c, centriol; e, ellipsoid); b, the optic nerve (nt, neurotubule; nf, neurofilament); and c, the optic tract (ll represents lateral lemniscus; ep, commissura posterior; tthy, tractus tectothalamicus and hypothalamicus cruciatus; and ft, fasciculus lateralis telencephale).

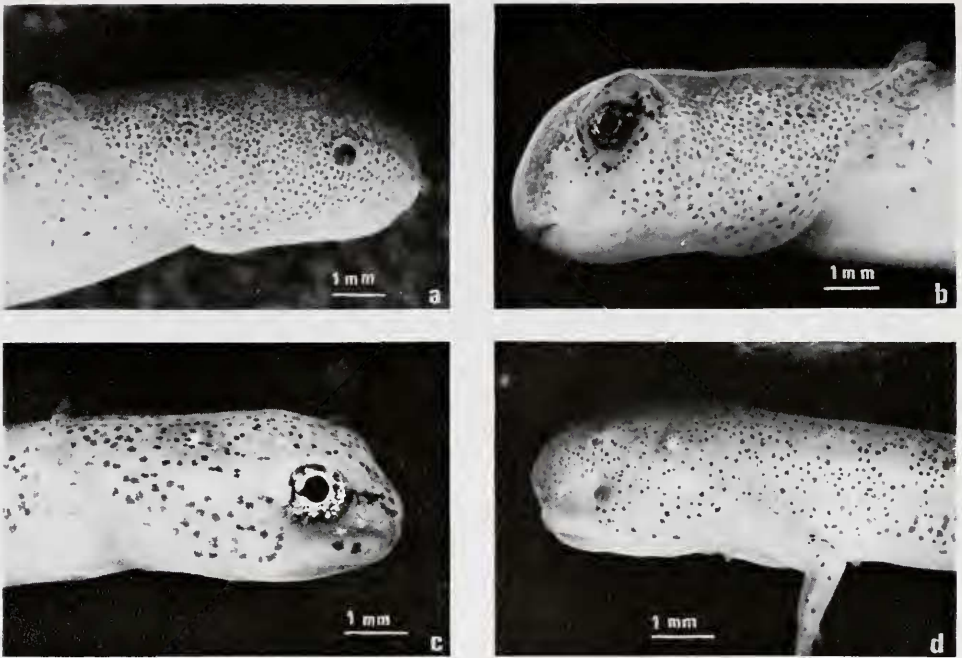


FIGURE 6. Macrophotographs of a: the *Proteus* larva (27 mm length); b, the larva (27 mm length) with a grafted *Euproctus* eye; c, the *Euproctus* larva (19 mm in length); d, the *Euproctus* larva (19 mm length) with a grafted *Proteus* eye.

Conservation of the primitive lumen of the lens, permanence of lytic processes, and the possibility of nucleolar segregation in the cells of the cornea all demonstrate that the involution of the epiblastic derivatives of the eye corresponds to the persistence of a cellular metabolic disturbance (normal but transitory during the ocular development of other vertebrates).

Biometrics can be used to confirm the cytological findings. It is clear that the curves (Fig. 4) represent the growth of the eye and the lens and satisfy respectively the functions: bx^α and $bx^\alpha xe^{-\gamma x}$. The growth pattern of the lens is of the same type as the one for the eye and needs only be differentiated by the complementary term $e^{-\gamma x}$, where γx can be considered as the expression of the involutive phenomenon superimposed upon the normal growth of the lens.

In summary, the slowness of the processes of growth and of involution (along with the modifications which accompany them) suggests that there is in the lens a state of relative equilibrium between the processes of synthesis and degeneration.

Photosensitive retina. The photosensitive retina is composed of a pigmented epithelium which appears poorly developed (Nguyen Legros and Durand, 1974).

The visual cells of *Proteus* (Fig. 5a) appear to be degenerated if compared to those of other Urodela. One can find, however, the elements essential to a photoreceptor: the cell body, the inner segment with mitochondria, and a basal centriole sending ciliary fibrils through a peduncle which supports the outer segment. The

existence of a peduncle shows that each very flattened stack of double membrane disks, containing some vesicles and some tubules, corresponds to a rudimentary photoreceptor. The existence of such photoreceptors has been denied by earlier workers.

Neural retina. The disposition of the neural retina is simple; the number of photoreceptive elements, which is about 2000 for an adult *Proteus*, is very low in comparison to 110,000 possessed by *Necturus* whose retina is regarded as poorly developed. The body of the receptor is joined at the base by the fiber of Henlé which terminates by dendritic expansions enclosing synaptic vesicles and synaptic ribbons. The external plexiform layer of *Proteus* is therefore built following the classic pattern. Similarly, the internal plexiform layer includes active zones on a level where the synaptic membranes separate the end buds of the bipolar cells and the dendrites of the ganglionic cells. Relationships between the various categories of nerve cells of the retina appear normal.

Optic tracts. The existence and continuity of the optic tracts have often been contested. The axons of the optic nerve issue from the multipolar cells of the retina, in a tenuous layer joined together in a nervous tract. On their extracranial pathway, the ophthalmic nerve and artery pass above the pterygoid, under the deep ophthalmic branch of the trigeminal nerve and above the cranial trabecula before penetrating the cranium by an optic foramen opening through the orbital lamella of the parietal bone. At this level, the diameter of the optic nerve is from 9–15 μm in the larva and from 10–30 μm in the adult.

The optic nerve enters the brain at the level of the supraoptic nucleus. It is enclosed in a stalk of glial cells, the lumen of which communicates with the optic recess of the third ventricle. The optic nerve is made up of nonmyelinated fibers of very thin diameter (Fig. 5b). For some of them sizes range between 0.10 and 0.60 μm , and they contain neurotubules while others are larger including both neurotubules and neurofilaments. The myelinated fibers are less numerous and their diameter can exceed one micrometer. Their sheaths are composed of ten to twenty lamellae. Some of these fibers terminate in the presynaptic prominence of the anterior accessory optic tract.

The optic fibers are bent back towards the chiasma. From each side of the chiasma a small number of fibers run towards the lateral anterior region of the mesencephalon, making up the axial marginal optic tract (Fig. 5c). The optic tectum of the adult is relatively less developed than that of the young animal and occasionally resembles the degenerated optic region found in cavernicolous fishes.

In summary, the optic tract of the young *Proteus* does not show any break in its continuity between the photoreceptors and the optic tectum. The connection of the organs necessary to transmission of information exists, and this appears to agree with the electrophysiological results of Zener (1971), at least as far as the eye is concerned.

It is evident from the study of the relationships between the eye and the orbit that certain peculiarities of the visual apparatus are related to modifications of its orbital adnexa, such as the elongation of the vessels, muscles and the optic nerve and the displacement of the optic nerve center towards the posterior region of cranium. These are anatomical modifications often connected with the elongation of the head and body in other forms.

EXPERIMENTS ON DEGENERATION OF THE EYE

Subterranean environment and microphthalmia

Lack of pigmentation and ocular degeneration are considered to be two essential characteristics of adaptation of an animal to cavernicolous life. This is true for the lack of pigmentation in *Proteus*. In fact, pigmentation appears at the beginning of development in the absence of all light stimulation. Young larvae maintained in light show melanophores and also yellowish chromatophores. Pigmentation is controlled thereafter by a physiological regulation in relation to conditions of light or darkness present in the environment of the animal. Accordingly, the lack of pigment in *Proteus* appears to be an adaptative character and not a degenerative feature.

The condition is other than that suggested by the term, "ocular degeneration." An adaptative relationship between the darkness underground and the disappearance of the eyes (which have become unnecessary) is accepted by a great number of authors (Gostejeva, 1949). In fact, this opinion only stands on a single experiment, still very debatable, made by Kammerer (1912).

Experiments were designed to find out if the presence of light will actually permit regeneration of eyes. Young specimens of *Proteus* were exposed to the action of daylight for 6.5 years. Others were exposed to artificial light of wavelengths between 600 and 1200 nm (i.e., within the red and infrared range of the spectrum). This continued for the first 10 years of age. After several years, individuals exposed to light become bluish-black. Over the same time, even if the eye is initially relatively well developed, the corneal ectoderm thickens, the lens diminishes in size and eventually disappears.

It is known that at the level of the photoreceptive cells, light exerts a certain action. However at the level of the entire visual apparatus, and within the limits of our experiments, it does not impede at all the manifestation of degenerative processes including the sinking in of the eye within the orbit, the disappearance of the lens, and the thickening of the dermal cornea.

Humoral conditions of ocular involution

The subterranean environment does not seem to have a determinant ontogenetic influence in transplant studies of microphthalmia. This problem was studied in order to test the hypothesis (see, for example, Hawes, 1946), that the eye of *Proteus* results from a stopping of development tied to the neotenic condition of the animal. Xenoplastic grafts have permitted us to follow, after determination of the field, the development of the eye of *Proteus* transplanted to a host in which the eyes develop normally. Conversely, the development of a foreign ocular field was followed in *Proteus* (Fig. 6).

The grafts between *Proteus* and a certain number of other species were rejected (*Triturus* spp.), but transplantations between *Proteus* and either *Pleurodeles waltlii* or *Euproctus asper* gave satisfactory results.

Implants of Proteus. The graft of the ocular field of *Proteus* on the lateral body wall of *Euproctus* or *Pleurodeles* developed only weakly. It is the same for

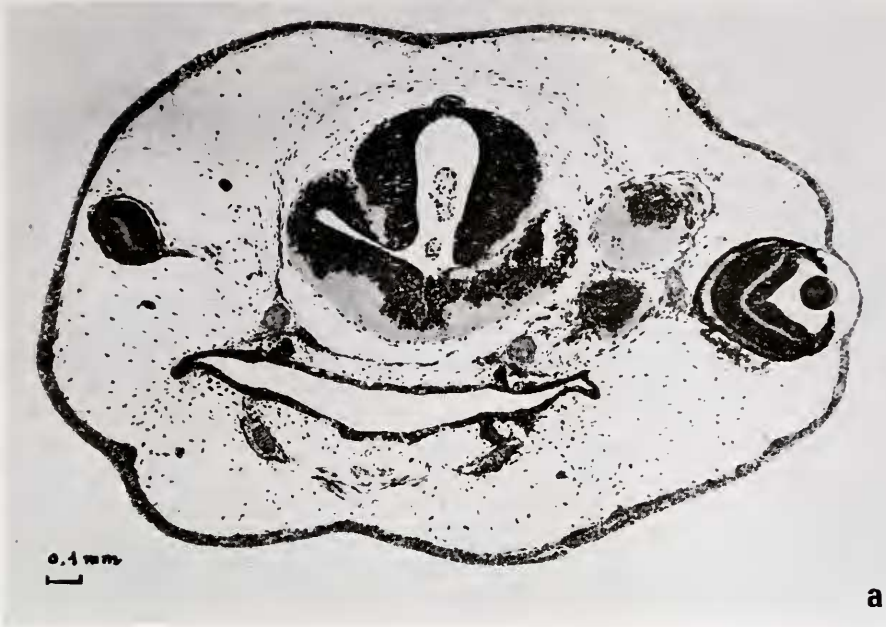


FIGURE 7. Micrographs of a: the *Euproctus* eye in the orbit of *Proteus* 33 days after transplantation; and b, the *Proteus* eye in the orbit of *Euproctus* 37 days after transplantation.

grafts to the orbits of these animals (Fig. 6d) in which each graft did not go beyond the stage of development normally attained by the eye of *Proteus*. The corresponding histological sections show the weak development of the ocular field of *Proteus* grafted in the orbit of *Euproctus* (Fig. 7b). After transplantation to a foreign host, the autodifferentiation of the ocular anlagen of *Proteus* does not permit it to build a normal ocular apparatus, but only the usual rudimentary eye.

Implants to Proteus. The xenoplastic graft on the lateral body wall of *Proteus* of an ocular field of *Pleurodeles* or *Euproctus*, shows that the field frequently differentiates in an atypical fashion. However, after the homotopic xenoplastic graft of an ocular field of *Pleurodeles* or *Euproctus* on *Proteus*, the graft (Fig. 6b) develops and is pigmented in the same way as it would in the donor orbit (Fig. 7a).

At the end of these experiments, one can say that the grafted eyes develop by autodifferentiation up to and including the time of degeneration. The development of the eye of *Proteus* appears therefore to be independent of the host on which it is grafted.

By a different technique, one can show that the factors responsible for the ocular involution do not have a hormonal origin (thyroxin) as earlier thought. In fact, it is impossible to demonstrate that thyroxin, in solution or by injection has any effect on the development of the eye of *Proteus*. For example, in the eye of animals kept in thyroxin and hydrocortisone solution for five months, with the upper limit of thyroxin being 10 mg/liter, there was only a slight thickening of the general skin.

Mechanism of autodifferentiation

After having shown that the ocular field of *Proteus* develops by autodifferentiation, we tried to demonstrate the mechanism. Reciprocal or cross-transplantation of the neural optic vesicle and its overlying ectoderm allowed the evaluation of the influence of the optic vesicle on the differentiation of a lens or of a cornea. The "inducing influence" of the optic vesicle of *Proteus* operates more slowly and more weakly than that of the optic vesicle of some other Urodela. Conversely, the ectoderm of *Proteus* subjected to a normal inducing action shows a certain "competence" in the differentiation both of a lens and of a cornea. However, this competence is less than that shown by the presumptive ectoderm of either *Pleurodeles* or *Euproctus*.

This can be confirmed by regeneration experiments after ablation of the eye (and particularly of the lens). The morphogenetic ability of the retina is exerted in such cases, later and more slowly in *Proteus*. From this we can conclude that the regulation of organogenesis of the eye unfolds as in the general case, but the reactions of the tissues are specifically weaker and slower than those in the epigeous Urodela.

DISCUSSION

It is worth remembering how much the problem of ocular degeneration of *Proteus* has interested generations of naturalists. Many regard this degeneration as a secondary adaptation to the subterranean environment; in contrast, others regard it as a neotenic developmental character or as an earlier preadaptation to a

cavernicolous life. One recent hypothesis is that of Hawes (1946), who considered the ocular reduction of *Proteus* as being dependent on a neotenic preadaptation secondarily reinforced by a subterranean life. In fact, it is very difficult to deduce any phylogenetic evolution for the ocular apparatus of *Proteus*, because the ontogeny of *Proteus* is such that ocular reduction does not depend upon these factors. However, all the anatomical and experimental evidence confirms that the determining factors of the regression are genetic.

The numerous convergences of form between *Proteus* and the other microphthalmic vertebrates such as *Typhlomolge* (= *Eurycea rathbuni* from the caves of Texas), suggest that they result from parallel eye evolution. If one compares the ocular reduction of *Proteus* to that observed in the case of cavernicolous fishes or other Urodela, one can get a clearer idea of the structure and development of these rudimentary eyes. The general pattern involves: slowness of development, instability of the embryonic eye ectoblastic derivatives, and synchrony of constructive with destructive ocular processes. The destructive events involve the cornea and lens initially, but can often lead to the apparent disappearance of the eye in the adult animal.

In the course of this involution, the processes of differentiation to cornea are less important than those of differentiation to teguments. The lens is greatly affected by persistence of lytic processes, which are stronger than those of normal development. The secondary degeneration affects the pigmented epithelium and the photoreceptors, with certain disorganization of the retinal structures and of the optic tractus and tectum.

The usual term "degenerate eye" is misleading. The rudimentary eyes in *Proteus*, and probably in other cave vertebrates, result from specific development and are to be considered as produced by a disturbance of normal ontogenic processes and of cellular metabolism.

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SUMMARY

The anatomy and development of the eye of *Proteus anguinus* are described. The relationships between organogenesis of the eye in embryos and larva and its involution in the young and the adult are discussed.

The availability (in breeding cultures) of a significant number of *Proteus* embryos (which are normally rare) allowed experimental analysis of the effects of light, xenoplastic differentiation and thyroid hormones on the development of the eye.

The results of this study suggest that development and involution of the eye of *Proteus* are controlled by genetic factors which are not greatly influenced by environment, and one can, therefore, consider the microphthalmia of *Proteus* as a relict characteristic which is the result of a specific development with disturbance of the normal ontogenic process.

LITERATURE CITED

- DURAND, J. P., 1971. Recherches sur l'appareil visuel du Protée, *Proteus anguinus* Laurenti, Urodèle hypogé. *Ann. Spelcol.*, **26**: 497-824.
- GOSTEJEVA, M. N., 1949. Nouvelles données sur la réduction des yeux chez les Protées. *C. R. Acad. Sci. U. R. S. S.*, **67**: 177-180.
- HAWES, R. S., 1946. On the eyes and reactions to light of *Proteus anguinus*. *Quart. J. Microsc. Sci. N. S.*, **86**: 1-53.
- KAMMERER, P., 1912. Experimente über fortpflanzung, farbe, augen und körperreduction bei *Proteus anguinus* Laurenti. *Archiv. Entwicklungsmech. Organismen*, **33**: 348-461.
- NGUYEN LEGROS, J. AND J. P. DURAND, 1974. Ultrastructure de l'épithélium pigmentaire rétinien du Protée, Urodèle cavernicole. Relations fonctionnelles avec les photorécepteurs. *Ann. Speleol.*, **4**: 671-674.
- VANDEL, A., 1966. Le Protée et sa place dans l'embranchement des Vertébrés. *Bull. Soc. Zool. Fr.*, **91**: 171-178.
- VANDEL, A., J. P. DURAND AND M. BOUILLON, 1964. Observations sur le développement du Protée, *Proteus anguinus* Laurenti (Batraciens, Urodèles). *C. R. Hebd. Séanc. Acad. Sci. Paris*, **259**: 4801-4804.
- VANDEL, A. AND J. P. DURAND, 1970. Le cycle vital du Protée. *C. R. Hebd. Séanc. Acad. Sci. Paris*, **270**: 2699-2701.
- ZENER, B., 1971. The light sensitivity of the cave salamander *Proteus anguinus* Laur. *Periodicum biologorum, Soc. Sci. Natur. Croatica*, **73A**: 16.